

Supporting Information

Discovery and Evaluation of *N*-Cyclopropyl-2,4-difluoro-5-((2-(pyridin-2-ylamino)thiazol-5-ylmethyl)amino) benzamide (BMS-605541), a Selective and Orally Efficacious Inhibitor of Vascular Endothelial Growth Factor Receptor-2 (VEGFR-2)

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I. General Experimental Details

All non-aqueous reactions were carried out under an argon or nitrogen atmosphere at room temperature, unless otherwise noted. All commercial reagents and anhydrous solvents were purchased from Aldrich and were used without further purification or distillation, unless otherwise stated. Analytical thin layer chromatography (tlc) was performed on EM Science silica gel 60 F₂₅₄ (0.25 mm). Compounds were visualized by UV light and/or stained with either *p*-anisaldehyde, potassium permanganate, or cerium molybdate solutions followed by heating. Flash column chromatography was performed on EM Science silica gel 60 (particle size of 40–63 μ m). Analytical high pressure liquid chromatography (HPLC) and LC-MS analyses were conducted using Shimadzu LC-10AS pumps and a SPD-10AV UV-vis detector set at 220 nm with the MS detection performed with a Micromass Platform LC spectrometer. Analytical HPLC analyses were performed using the following conditions:

Method A: phenom-prime S5 C18 4.6 \times 30 mm column, solvent A: 10% MeOH-90% H₂O-0.1% TFA, solvent B: 90% MeOH-10% H₂O-0.1% TFA, flow rate = 4 mL/min; linear gradient time = 2 min; start %B = 0, final %B = 100, stop time 3 min; or

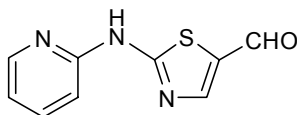
Method B: phenomenex Su C18 4.6 \times 50 mm column, solvent A: 10% MeOH-90% H₂O-0.1% TFA, solvent B: 90% MeOH-10% H₂O-0.1% TFA, flow rate = 4 mL/min; linear gradient time = 4 min; start %B = 0, final %B = 100, stop time 5 min.

Preparative reverse phase (RP) HPLC was performed using two Shimadzu LC-8A pumps, a SPD-10AV UV-vis detector set at 220 nm, and a YMC C18 ODS-A 5 μ m, 20 \times 100 mm column (eluting at 20 mL/min with a 12 minute gradient of 0-100% B where solvent A = 10% MeOH-90% H₂O-0.1% TFA and solvent B = 90% MeOH-10% H₂O-0.1% TFA). Following preparative HPLC purification, the fractions containing the desired product were concentrated under reduced pressure to remove the MeOH, neutralized with saturated aqueous NaHCO₃ to pH 7 and extracted with ethyl

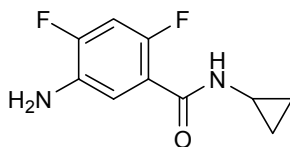
acetate (3×5 mL). The organic layers were dried (Na_2SO_4) and concentrated *in vacuo* to afford the desired compounds.

NMR (^1H and ^{13}C) spectra were recorded on one of the following instruments: JEOL GSX-500 MHz, or Bruker ARX-400 MHz spectrometers and calibrated using an internal reference. High-resolution mass spectra (HMRS) were recorded on a JEOL SX102 mass spectrometer. Elemental analyses were performed by Robertson Microlit Laboratories and the results obtained are within $\pm 0.4\%$ of the theoretical values.

II. Detailed Experimental Procedures for the Preparation of Compound 14 (BMS-605541)

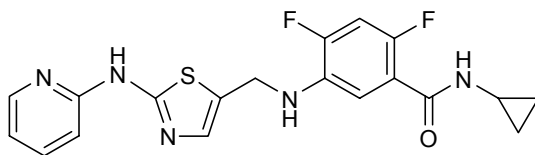


2-(Pyridin-2-ylamino)thiazole-5-carbaldehyde (16). A mixture of 1-(pyridin-2-yl)thiourea¹ (**15**, 1.53 g, 10 mmol), 2-bromo-3-hydroxypropenal² (1.51 g, 10 mmol) and sodium acetate (1.5 g, 18 mmol) in acetone (20 mL) was refluxed for 2 h. The mixture was cooled to 25 °C and poured into water. The solid was collected, washed with water and dried under high vacuum overnight to afford 2-(pyridin-2-ylamino)thiazole-5-carbaldehyde (**16**, 1.35 g, 66%) as a solid: Anal. RP-HPLC t_R = 1.29 min (method A, purity 97%), t_R = 2.66 min (method B, purity 98%); ^1H NMR (500 MHz, $\text{DMSO}-d_6$) δ 12.09 (s, 1H), 9.86 (s, 1H), 8.41 (d, 1H, J = 5.2 Hz), 8.32 (s, 1H), 7.79–7.74 (m, 1H), 7.16 (d, 1H, J = 8.8 Hz), 7.06 (dd, 1H, J = 5.2, 7.1 Hz); ^{13}C NMR (125 MHz, $\text{DMSO}-d_6$) δ 182.9, 165.4, 151.6, 150.4, 146.4, 138.3, 130.2, 117.4, 111.8; MS (ESI) m/z 206 ($\text{M} + \text{H}$)⁺; Anal. ($\text{C}_9\text{H}_7\text{N}_3\text{OS}$) Calcd.: C, 52.67; H, 3.44; N, 20.47; S, 15.62. Found: C, 52.44; H, 3.18; N, 20.52; S, 15.55.



5-Amino-*N*-cyclopropyl-2,4-difluorobenzamide (18). A solution of 2,5-difluoro-4-nitrobenzoic acid³ (2.03 g, 10 mmol) in thionyl chloride (10 mL) was refluxed for 3 h. The excess thionyl chloride was removed *in vacuo* and the residue was dissolved in CH₂Cl₂ (20 mL). The solution was cooled to –40 °C and cyclopropylamine (570 mg, 10 mmol) was added, followed by triethylamine (2.02 g, 20 mmol). The mixture was stirred at –40 °C for 1 h and quenched by the addition of 1 N aqueous HCl solution (10 mL). The organic layer was separated, washed with 1 N HCl solution (10 mL) and saturated aqueous NaHCO₃ solution (10 mL), dried (Na₂SO₄) and concentrated *in vacuo* to afford *N*-cyclopropyl-2,4-difluoro-5-nitrobenzamide (**17**, 1.95 g, 80%) as a solid, which was used directly in the subsequent reaction. Anal. RP-HPLC: t_R = 2.43 min (method B, purity 98%); ¹H NMR (500 MHz, CDCl₃) δ 8.93 (t, 1H, J = 8.3 Hz), 7.13 (t, 1H, J = 10.5 Hz), 6.68 (brs, 1H), 2.97–2.94 (m, 1H), 0.94–0.89 (m, 2H), 0.67–0.61 (m, 2H).

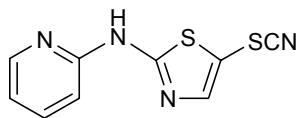
A solution of compound **17** (1.0 g, 4.1 mmol) in an ethyl acetate – ethanol mixture (1:1, 15 mL) at 25 °C under Ar was treated with 10% palladium on carbon (0.1 g). The reaction mixture was stirred under a blanket of H₂ (1 atm) for 2 h and filtered through a pad of Celite[®] (EtOH). The solvents were removed *in vacuo* to afford 5-amino-*N*-cyclopropyl-2,4-difluorobenzamide (**18**, 0.85 g, 97%) as a white solid: Anal. RP-HPLC: t_R = 1.67 min (method B, purity 98%); ¹H NMR (400 MHz, DMSO-*d*₆) δ 8.16 (brs, 1H), 7.09 (dd, 1H, J = 11.0, 10.4 Hz), 6.96 (dd, 1H, J = 10.0, 7.2 Hz), 5.16 (brs, 2H), 2.82–2.77 (m, 1H), 0.71–0.64 (m, 2H), 0.54–0.48 (m, 2H); ¹³C NMR (125 MHz, DMSO-*d*₆) δ 165.1, 151.6 (dd, J = 247, 12 Hz), 149.3 (dd, J = 242, 11 Hz), 133.2 (d, J = 16 Hz), 120.5 (d, J = 16 Hz), 116.1, 104.3 (dd, J = 28, 23 Hz), 23.3, 6.1 (2C); MS (ESI) m/z 213 (M + H)⁺; Anal. (C₁₀H₁₀F₂N₂O) Calcd.: C, 56.60; H, 4.75; F, 17.91; N, 13.20. Found: C, 56.47; H, 4.55; F, 18.03; N, 13.21.



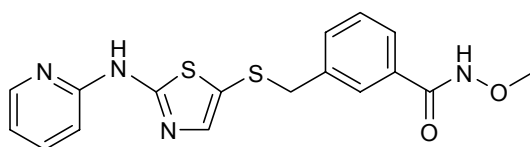
***N*-Cyclopropyl-2,4-difluoro-5-((2-(pyridin-2-ylamino)thiazol-5-ylmethyl)amino)benzamide (14, BMS-605541).** A mixture of compound **16** (210 mg, 1.0 mmol) and compound **18** (250 mg, 1.2 mmol) in TFA/CH₂Cl₂ (1:1, 4 mL) was stirred at 25 °C for 20 min and then treated with triethylsilane (0.4 mL). The resulting solution was stirred at ambient temperature for 1 h and concentrated *in vacuo*. The residue was neutralized with disodium phosphate solution (5 mL). The precipitate that formed was collected, washed with water and acetone, and dried overnight to afford **14** (300 mg, 74%) as a white solid: Anal. RP-HPLC t_R = 1.31 min (method A, purity 99%), t_R = 2.90 min (method B, purity 99%); ¹H NMR (500 MHz, DMSO-*d*₆) δ 11.06 (s, 1H), 8.23 (d, 1H, J = 4.6 Hz), 8.11 (s, 1H), 7.65 (t, 1H, J = 7.3 Hz), 7.24 (s, 1H), 7.13 (t, 1H, J = 10.9 Hz), 7.02 (d, 1H, J = 8.3 Hz), 6.95 (dd, 1H, J = 9.7 Hz), 6.87 (d, 1H, J = 6.2 Hz), 6.08 (s, 1H), 4.44 (d, 2H, J = 5.2 Hz), 2.83–2.74 (m, 1H), 0.72–0.59 (m, 2H), 0.53–0.46 (m, 2H); ¹³C NMR (125 MHz, DMSO-*d*₆) δ 164.5, 159.1, 151.7, 151.1 (dd, J = 245, 12 Hz), 150.0 (dd, J = 241, 11 Hz), 146.3, 137.7, 135.1, 132.7 (d, J = 13 Hz), 127.4, 119.9 (d, J = 15 Hz), 115.7, 112.2, 110.6, 103.8 (dd, J = 27, 26 Hz), 39.3, 22.9, 5.71 (2C); HRMS for C₁₉H₁₇F₂N₅OS (M + H)⁺, calcd: 402.1200, found: 402.1194; Anal. (C₁₉H₁₇F₂N₅OS) Calcd.: C, 56.84; H, 4.26; F, 9.46; N, 17.44; S, 7.98;. Found: C, 56.78; H, 4.17; F, 9.60; N, 17.39; S, 8.05.

The UV Purity (Homogeneity Index) of **14** was determined to be > 99.5% (t_R = 21.53 min) by HPLC (0–100% B) at 220 nm using a 42 minute gradient (YMC-PackPro C18 3μ 4.6 × 150 mm column at 25 °C; solvent A: 80% H₂O-20% MeCN with 25 mM ammonium acetate, solvent B: 80% MeCN-20% H₂O with 25 mM ammonium acetate; flow rate = 1 mL/min).

III. Experimental Procedures and Characterization Data for Analogues 3–13



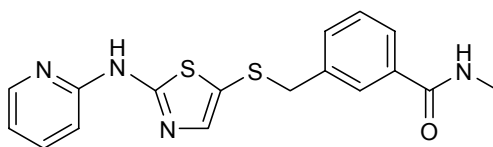
***N*-(5-Thiocyanatothiazol-2-yl)pyridin-2-amine.** To a suspension of *N*-(thiazol-2-yl)pyridin-2-amine⁴ (0.65 g, 3.7 mmol) and ammonium thiocyanate (0.56 g, 7.3 mmol) in MeOH (20 mL) at 0 °C was added bromine (0.65 g, 0.21 mL, 4.0 mmol) dropwise over 10 min. The reaction mixture was warmed to 25 °C, and the resulting homogeneous solution was stirred at ambient temperature for 1 h. The reaction mixture was poured into water (15 mL), extracted with EtOAc (3 × 15 mL) and CHCl₃ (3 × 15 mL). The organic extracts were concentrated *in vacuo* and the resulting solid was triturated with MeOH / CH₂Cl₂ (1:1, 3 × 5 mL) to afford *N*-(5-thiocyanatothiazol-2-yl)pyridin-2-amine (0.65 g, 76%) as an off-white solid: Anal. RP-HPLC: *t*_R = 1.54 min (method A, purity 98%); *t*_R = 2.89 min (method B, purity 98%); ¹H NMR (400 MHz, DMSO-*d*₆) δ 11.94 (s, 1H), 8.38 (d, 1H, *J* = 4.1 Hz), 7.89 (s, 1H), 7.79 (t, 1H, *J* = 7.7 Hz), 7.10 (d, 1H, *J* = 8.2 Hz), 7.04 (t, 1H, *J* = 6.4 Hz); ¹³C NMR (100 MHz, DMSO-*d*₆) δ 164.2, 150.6, 148.2, 146.2, 138.5, 117.1, 112.0, 111.2, 102.7; HRMS for C₉H₆N₄S₂ (M + H)⁺, calcd: 235.0114, found: 235.0102.



***N*-Methoxy-3-((2-(pyridin-2-ylamino)thiazol-5-ylthio)methyl)benzamide (3).** To a solution of *N*-(5-thiocyanatothiazol-2-yl)pyridin-2-amine (0.16 g, 0.68 mmol) in EtOH-THF (1:1, 8 mL) was added NaBH₄ (31 mg, 0.82 mmol). The reaction mixture was stirred for 25 min and treated with 3-(chloromethyl)benzoic acid (0.13 g, 0.88 mmol). The resulting solution was stirred for 2 h, treated dropwise with acetone (1 mL) and concentrated *in vacuo*. The residue was dissolved in CH₂Cl₂ (5 mL) and pH 7 phosphate buffer (5 mL), and the layers were separated. The aqueous layer was extracted with CH₂Cl₂ (3 × 5 mL) and the combined organic extracts were dried (Na₂SO₄) and concentrated *in vacuo*.

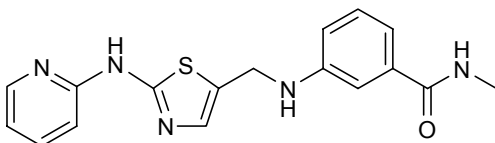
The crude intermediate, 3-((2-(pyridin-2-ylamino)thiazol-5-ylthio)methyl)benzoic acid (12 mg, 5.1%) was used in the subsequent step without purification. MS (ESI) m/z 344 ($M + H$)⁺.

The above intermediate (12 mg, 35 μ mol) was dissolved in DMF (0.5 mL), treated with methoxylamine-hydrochloride (3.5 mg, 42 μ mol), HOBt (4.7 mg, 35 μ mol), *N*-methylpyrrolidine (6.0 mg, 7.3 μ L, 70 μ mol) and EDCI (10 mg, 52 μ mol) at 25 °C. The reaction mixture was stirred at ambient temperature for 16 h and purified directly by preparative HPLC to afford compound **3** (3.1 mg, 24%) as a white film: Anal. RP-HPLC: t_R = 1.35 min (method A, purity 90%), 2.88 min (method B, purity 89%); ¹H NMR (400 MHz, CD₃OD) δ 8.33 (d, 1H, J = 5.4 Hz), 8.00 (t, 1H, J = 7.6 Hz), 7.67–7.58 (m, 2H), 7.44–7.39 (m, 2H), 7.24 (s, 1H), 7.23–7.17 (m, 2H), 4.04 (s, 2H), 3.76 (s, 3H); MS (ESI) m/z 373 ($M + H$)⁺.

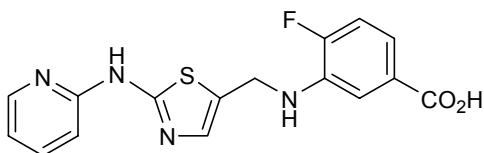


***N*-Methyl-3-((2-(pyridin-2-ylamino)thiazol-5-ylthio)methyl)benzamide (4).** To a solution of *N*-(5-thiocyanatothiazol-2-yl)pyridin-2-amine (34 mg, 0.15 mmol) in EtOH-THF (1:1, 4 mL) was added NaBH₄ (8.3 mg, 0.22 mmol). The reaction mixture was stirred for 25 min and treated with 3-(chloromethyl)-*N*-methylbenzamide⁵ (35 mg, 0.19 mmol). The resulting solution was stirred for 2 h, treated dropwise with acetone (0.5 mL) and concentrated *in vacuo*. The residue was dissolved in EtOAc (2 mL) and pH 7 phosphate buffer (2 mL), and the layers were separated. The aqueous layer was extracted with EtOAc (3 \times 3 mL) and the combined organic extracts were dried (Na₂SO₄) and concentrated *in vacuo*. The residue was purified using a Chromatotron (1 mm SiO₂ GF rotor, 2–5% MeOH/CHCl₃ gradient elution) to afford **4** (10 mg, 20%) as a white solid: Anal. RP-HPLC: t_R = 1.46 min (method A, purity 98%), t_R = 2.98 min (method B, purity 96%); ¹H NMR (400 MHz, DMSO-*d*₆) δ

11.40 (brs, 1H), 8.40 (brs, 1H), 8.31–8.27 (m, 1H), 7.82–7.67 (m, 3H), 7.46–7.30 (m, 2H), 7.16 (s, 1H), 7.12–7.04 (m, 1H), 7.01–6.93 (m, 1H), 4.01 (s, 2H), 2.76 (s, 3H); MS (ESI) m/z 357 ($M + H$)⁺.



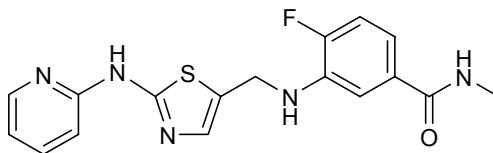
***N*-Methyl-3-((2-(pyridin-2-ylamino)thiazol-5-ylmethyl)amino)benzamide (5).** Prepared in a similar manner to compound **14**, using aldehyde **16** and 3-amino-*N*-methylbenzamide. Anal. RP-HPLC: t_R = 1.06 min (method A, purity 95%); ¹H NMR (400 MHz, CD₃OD) δ 8.13 (d, 1H, J = 4.3 Hz), 7.50 (t, 1H, J = 8.3 Hz), 7.13 (s, 1H), 7.12–6.98 (m, 2H), 6.96–6.89 (m, 1H), 6.86 (d, 1H, J = 8.4 Hz), 6.78–6.69 (m, 2H), 4.36 (s, 2H), 2.79 (s, 3H); MS (ESI) m/z 340 ($M + H$)⁺.



4-Fluoro-3-((2-(pyridin-2-ylamino)thiazol-5-ylmethyl)amino)benzoic acid. To a suspension of 2-(pyridin-2-ylamino)thiazole-5-carbaldehyde (**16**, 1.0 g, 4.9 mmol) in methylene chloride (20 mL) at 25 °C was added 3-amino-4-fluorobenzoic acid (0.95 g, 6.1 mmol), TFA (5 mL) and triethylsilane (2.3 mL, 1.7 g, 15 mmol). The reaction mixture was stirred at ambient temperature for 4 h and concentrated *in vacuo*. The resulting slurry was triturated with MeOH to afford 4-fluoro-3-((2-(pyridin-2-ylamino)thiazol-5-ylmethyl)amino)benzoic acid (1.53 g, 91%) as a light yellow solid: Anal. RP-HPLC: t_R = 1.32 min (method A, purity 97%), t_R = 2.15 min (method B, purity 95%); ¹H NMR (400 MHz, DMSO-*d*₆) δ 11.81 (brs, 1H), 8.29 (d, 1H, J = 4.4 Hz), 7.76 (t, 1H, J = 7.4 Hz), 7.74 (s, 1H), 7.37 (d, 1H, J = 8.3 Hz), 7.23–7.19 (m, 1H), 7.17–7.10 (m, 2H), 7.00 (t, 1H, J = 6.0 Hz), 4.52 (s, 2H); ¹³C NMR (125 MHz, DMSO-*d*₆) δ 166.8, 159.2, 154.4, 152.4, 150.7, 145.6, 138.3, 135.6 (d, J = 14 Hz), 132.3,

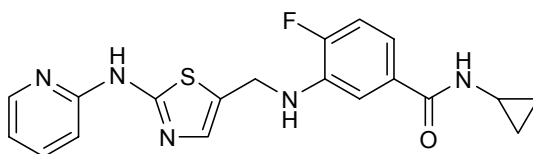
127.4 (d, $J = 70$ Hz), 117.9 (d, $J = 8$ Hz), 116.4, 114.2, (d, $J = 23$ Hz), 113.0, 111.1, 54.6; HRMS for $C_{16}H_{13}FN_4O_2S$ ($M + H$)⁺, calcd: 345.0822, found: 345.0828.

Compounds **6**, **7**, and **9-12** were prepared by treating a solution of 4-fluoro-3-((2-(pyridin-2-ylamino)thiazol-5-ylmethyl)amino)benzoic acid (1 eq) and the appropriate primary amine (2 eq) in DMF or THF with BOP reagent (benzotriazol-1-yloxy-tris(trimethylamino)phosphonium hexafluorophosphate, 2 eq). The reaction mixture was stirred at ambient temperature for 1–2 h. The mixture was then diluted with EtOAc, washed twice with saturated aqueous $NaHCO_3$ solution, dried (Na_2SO_4) and concentrated *in vacuo*. The residue was then purified by flash column chromatography (SiO_2 , 0–5% MeOH/ CH_2Cl_2 gradient elution) or preparative HPLC to afford the desired analogue.



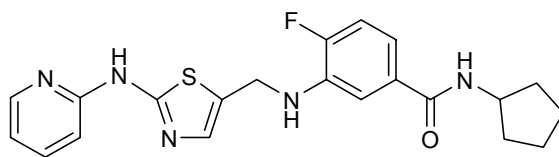
4-Fluoro-N-methyl-3-((2-(pyridin-2-ylamino)thiazol-5-ylmethyl)amino)benzamide (6).

Anal. RP-HPLC: $t_R = 1.17$ min (method A, purity 98%), $t_R = 2.33$ min (method B, purity 98%); 1H NMR (400 MHz, $CDCl_3$) δ 9.60 (brs, 1H), 8.33 (d, 1H, $J = 4.4$ Hz), 7.62 (t, 1H, $J = 8.5$ Hz), 7.36 (s, 1H), 7.36–7.28 (m, 1H), 7.02–6.98 (m, 2H), 6.91–6.86 (m, 2H), 6.10–6.03 (m, 1H), 4.55 (d, 2H, $J = 5.6$ Hz), 4.38–4.32 (m, 1H), 2.99 (d, 3H, $J = 4.8$ Hz).



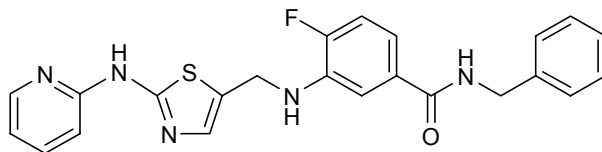
***N*-Cyclopropyl-4-fluoro-3-((2-(pyridin-2-ylamino)thiazol-5-ylmethyl)amino)benzamide (7).**

Anal. RP HPLC: t_R = 1.37 min (method A, purity 97%); t_R = 2.48 min (method B, purity 98%); ^1H NMR (500 MHz, $\text{DMSO-}d_6$) δ 11.10 (s, 1H), 8.26 (d, 1H, J = 4.0 Hz), 8.23 (d, 1H, J = 5.3 Hz), 7.66 (t, 1H, J = 6.6 Hz), 7.30–7.27 (m, 1H), 7.26 (s, 1H), 7.10–7.00 (m, 3H), 6.88–6.86 (m, 1H), 6.25–6.19 (m, 1H), 4.49 (d, 2H, J = 6.2 Hz), 2.78 (m, 1H), 0.67 (m, 2H), 0.54 (m, 2H); HRMS for $\text{C}_{19}\text{H}_{18}\text{FN}_5\text{OS}$ ($\text{M} + \text{H}$) $^+$, calcd: 384.1294, found: 384.1310.



***N*-Cyclopentyl-4-fluoro-3-((2-(pyridin-2-ylamino)thiazol-5-ylmethyl)amino)benzamide (9).**

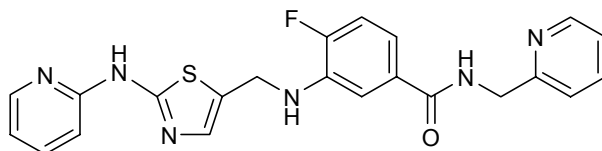
Anal. RP-HPLC: t_R = 1.49 min (method A, purity 98%), t_R = 2.95 min (method B, purity 97%); ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ 11.11 (s, 1H), 8.23 (d, 1H, J = 4.9 Hz), 8.07 (t, 1H, J = 7.2 Hz), 7.67 (t, 1H, J = 8.7 Hz), 7.31–7.29 (m, 1H), 7.28 (s, 1H), 7.07 (d, 2H, J = 8.7 Hz), 7.02 (d, 1H, J = 8.3 Hz), 6.89 (dd, 1H, J = 6.9, 6.4 Hz), 6.22–6.18 (m, 1H), 4.50 (s, 2H), 4.21–4.15 (m, 1H), 1.92–1.83 (m, 2H), 1.77–1.68 (m, 2H), 1.59–1.47 (m, 4H); HRMS for $\text{C}_{21}\text{H}_{22}\text{FN}_5\text{OS}$ ($\text{M} + \text{H}$) $^+$, calcd: 412.1607, found: 412.1609.



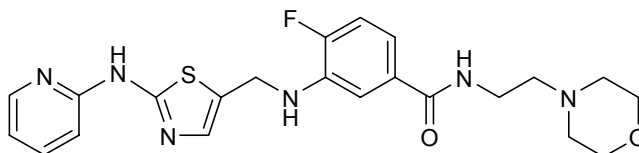
***N*-Benzyl-4-fluoro-3-((2-(pyridin-2-ylamino)thiazol-5-ylmethyl)amino)benzamide (10).**

Anal. RP-HPLC: t_R = 1.48 min (method A, purity 98%), t_R = 2.66 min (method B, purity 98%); ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ 11.09 (s, 1H), 8.90 (t, 1H, J = 5.9 Hz), 8.21 (dd, 1H, J = 5.0, 1.0 Hz), 7.66 (t, 1H, J = 8.7 Hz), 7.38 (d, 1H, J = 8.9 Hz), 7.32–7.29 (m, 5H), 7.28–7.24 (m, 1H), 7.16–7.10 (m, 2H),

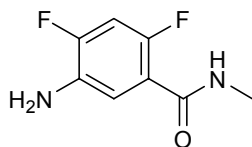
7.02 (d, 1H, $J = 8.4$ Hz), 6.87 (dd, 1H, $J = 6.7$ Hz), 6.29–6.26 (m, 1H), 4.50 (d, 2H, $J = 6.1$ Hz), 4.46 (d, 2H, $J = 5.9$ Hz); HRMS for $C_{23}H_{20}FN_5OS$ ($M + H$)⁺, calcd: 434.1451, found: 434.1446.



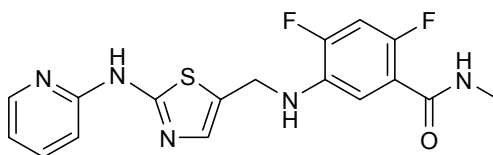
4-Fluoro-3-((2-(pyridin-2-ylamino)thiazol-5-ylmethyl)amino)-N-(pyridin-2-ylmethyl)benzamide (11). Anal. RP-HPLC: $t_R = 1.10$ min (method A, purity 98%), $t_R = 2.66$ min (method B, purity 98%); 1H NMR (400 MHz, DMSO- d_6) δ 11.12 (s, 1H), 9.04 (t, 1H, $J = 4.2$ Hz), 8.58 (d, 1H, $J = 5.0$ Hz), 8.29 (d, 1H, $J = 5.0$ Hz), 7.80 (t, 1H, $J = 7.6$ Hz), 7.74 (t, 1H, $J = 7.6$ Hz), 7.45 (d, 1H, $J = 10.3$ Hz), 7.38 (s, 1H), 7.37–7.31 (m, 2H), 7.24–7.21 (m, 2H), 7.09 (d, 1H, $J = 8.3$ Hz), 6.98–6.92 (m, 1H), 6.41–6.35 (m, 1H), 4.61 (d, 2H, $J = 5.9$ Hz), 4.58 (d, 2H, $J = 5.8$ Hz); HRMS for $C_{22}H_{19}FN_6OS$ ($M + H$)⁺, calcd: 435.1403, found: 435.1384.



4-Fluoro-N-(2-morpholinoethyl)-3-((2-(pyridin-2-ylamino)thiazol-5-ylmethyl)amino)benzamide (12). Anal. RP-HPLC: $t_R = 0.95$ min (method A, purity 98%), $t_R = 1.83$ min (method B, purity 99%); 1H NMR (500 MHz, DMSO- d_6) δ 11.09 (s, 1H), 8.28–8.20 (m, 2H), 7.66 (t, 1H, $J = 8.6$ Hz), 7.29 (d, 1H, $J = 7.6$ Hz), 7.28 (s, 1H), 7.12–7.01 (m, 3H), 6.88 (d, 1H, $J = 6.6$ Hz), 6.28–6.26 (m, 1H), 4.49 (d, 2H, $J = 5.9$ Hz), 3.57–3.54 (m, 4H), 3.37–3.33 (m, 2H), 2.47–2.40 (m, 6H); HRMS for $C_{22}H_{25}FN_6O_2S$ ($M + H$)⁺, calcd: 457.1822, found: 457.1834.

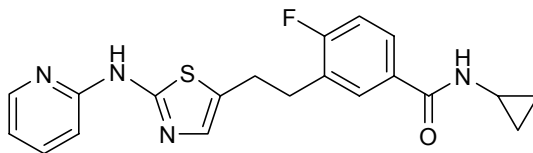


5-Amino-2,4-difluoro-*N*-methylbenzamide. Prepared in a similar manner as compound **18**, substituting methylamine for cyclopropylamine. Anal. RP-HPLC: t_R = 0.45 min (method A, purity 97%); t_R = 0.82 min (method B, purity 97%); ^1H NMR (400 MHz, CD_3OD) δ 7.10 (dd, 1H, J = 7.3, 7.4 Hz), 6.82 (t, 1 H, J = 10.7 Hz), 2.78 (s, 3H); ^{13}C NMR (100 MHz, CD_3OD) δ 166.9, 154.6 (dd, J = 248, 10 Hz), 152.3 (dd, J = 245, 12 Hz), 134.1 (d, J = 14 Hz), 119.6 (t, J = 10 Hz), 118.2, 105.0 (dd, J = 26, 23 Hz), 26.9; HRMS for $\text{C}_8\text{H}_8\text{F}_2\text{N}_2\text{O}$ ($\text{M} + \text{H}$) $^+$, calcd: 187.0684, found: 187.0679.



2,4-Difluoro-*N*-methyl-5-((2-(pyridin-2-ylamino)thiazol-5-ylmethyl)amino)benzamide (13).

Prepared in a similar manner as compound **14**, using 2-(pyridin-2-ylamino)thiazole-5-carbaldehyde (**16**) and 5-amino-2,4-difluoro-*N*-methylbenzamide. Anal. RP-HPLC: t_R = 1.25 min (method A, purity 97%), t_R = 2.40 min (method B, purity 98%); ^1H NMR (400 MHz, $\text{DMF}-d_7$) δ 11.07 (s, 1H), 8.30 (d, 1H, J = 4.1 Hz), 7.93–7.89 (m, 1H), 7.71 (t, 1H, J = 8.5 Hz), 7.32 (s, 1H), 7.28 (dd, 1H, J = 9.7 Hz), 7.18–7.12 (m, 2H), 6.92–6.89 (m, 1H), 6.14–6.09 (m, 1H), 4.59 (d, 2H, J = 5.8 Hz), 2.86 (d, 3H, J = 4.4 Hz); HRMS for $\text{C}_{17}\text{H}_{15}\text{F}_2\text{N}_5\text{OS}$ ($\text{M} + \text{H}$) $^+$, calcd: 376.1044, found: 376.1050.



***N*-Cyclopropyl-4-fluoro-3-(2-(2-(pyridin-2-ylamino)thiazol-5-yl)ethyl)benzamide (8).** A mixture of 3-bromo-4-fluorobenzoic acid (1.1 g, 5.1 mmol, Combi-Blocks Inc), cyclopropylamine (0.30 g, 5.3 mmol), BOP reagent (2.5 g, 5.7 mmol) and triethylamine (1 mL) in DMF (15 mL) was stirred at 25 °C for 1 h. Water (50 mL) was added to the reaction mixture and the resulting precipitate was collected on a Büchner funnel. The solid was washed with water and dried overnight to afford 3-bromo-*N*-cyclopropyl-4-fluorobenzamide (1.1 g, 85%), which was used directly in the subsequent reaction.

A solution of 3-butenal diethyl acetal (140 mg, 0.97 mmol) and 9-BBN (2.2 mL, 0.5 M in THF, 1.1 mmol) was stirred at 25 °C for 1 h and concentrated *in vacuo*. The residue was dissolved in benzene-ethanol (2:1, 3 mL) and treated with 3-bromo-*N*-cyclopropyl-4-fluorobenzamide (130 mg, 0.60 mmol), Pd(PPh₃)₄ (40 mg) and aqueous sodium carbonate solution (1 mL, 2 M). The reaction mixture was stirred at 80 °C under argon for 2 h, cooled to room temperature, and diluted with ethyl acetate (5 mL) and water (5 mL). The layers were separated, and the organics were dried over MgSO₄ and concentrated *in vacuo*. The residue was purified by flash chromatography (SiO₂; 3:1 hexanes / ethyl acetate) to afford *N*-cyclopropyl-3-(4,4-diethoxybutyl)-4-fluorobenzamide (151 mg, 93%) as an oil: ¹H NMR (500 MHz, CDCl₃) δ 7.62 (dd, 1 H, *J* = 7.5, 2.2 Hz), 7.58–7.54 (m, 1H), 7.00 (t, 1H, *J* = 9.0 Hz), 6.46 (s, 1H), 4.49 (t, 1H, *J* = 5.5 Hz), 3.62–3.49 (m, 4H), 2.69–2.67 (m, 2H), 1.69–1.65 (m, 4H), 1.21–1.18 (m, 6H), 0.88–0.84 (m, 2H), 0.65–0.61 (m, 2H).

A solution of *N*-cyclopropyl-3-(4,4-diethoxybutyl)-4-fluorobenzamide (151 mg, 0.47 mmol) and hydrochloric acid (0.2 mL, 3 N in water) in acetone (1 mL) was stirred at 25 °C for 1 h. The mixture was neutralized with sodium bicarbonate solution and extracted with ethyl acetate (3 × 5 mL). The organic extracts were dried over MgSO₄ and concentrated *in vacuo* to afford *N*-cyclopropyl-4-fluoro-3-(4-oxobutyl)benzamide (110 mg, 94%) as an oil, which was sufficiently pure to use in the subsequent reaction. ¹H NMR (500 MHz, CDCl₃) δ 9.76 (s, 1H), 7.63 (dd, 1H, *J* = 7.5, 2.2 Hz), 7.60–7.56 (m, 1H),

7.03 (t, 1H, $J = 9.0$ Hz), 6.47 (s, 1H), 2.89–2.85 (m, 1H), 2.69 (t, 2H, $J = 7.5$ Hz), 2.51–2.45 (m, 2H), 1.98–1.94 (m, 2H), 0.89–0.83 (m, 2H), 0.65–0.61 (m, 2H).

A mixture of *N*-cyclopropyl-4-fluoro-3-(4-oxobutyl)benzamide (110 mg, 0.44 mmol) and copper(II) bromide (150 mg, 0.67 mmol) in acetonitrile (1 mL) was stirred at 25 °C for 4 h. The reaction mixture was diluted with ethyl acetate (10 mL), washed with brine, dried over MgSO_4 and concentrated *in vacuo*. The resulting residue was dissolved in acetic acid (0.5 mL) and treated with compound **15** (67 mg, 0.44 mmol) and sodium acetate (100 mg, 1.2 mmol). The reaction mixture was stirred at 100 °C for 1 h, cooled to room temperature and neutralized with aqueous sodium bicarbonate solution. The mixture was extracted with ethyl acetate (3×5 mL) and the organics were concentrated *in vacuo*. The residue was purified by flash chromatography (SiO_2 , 1:1 ethyl acetate/hexanes) to afford compound **8** (17 mg, 10%) as a solid: Anal. RP-HPLC: $t_R = 1.58$ min (method A, purity 97%); ^1H NMR (500 MHz, $\text{DMSO}-d_6$) δ 11.03 (s, 1H), 8.48 (d, 1H, $J = 3.5$ Hz), 8.26 (d, 1H, $J = 5.3$ Hz), 7.67 (t, 1H, $J = 6.6$ Hz), 7.24 (t, 1H, $J = 9.35$ Hz), 7.04 (s, 1H), 7.02 (d, 1H, $J = 8.25$ Hz), 6.91–6.85 (m, 1H), 3.01 (t, 2H, $J = 7.7$ Hz), 2.95 (t, 2H, $J = 7.7$ Hz), 2.85–2.80 (m, 1H), 0.89–0.83 (m, 2H), 0.58–0.54 (m, 2H); MS (ESI) m/z 383 ($\text{M} + \text{H}$) $^+$.

IV. Table of Combustion Analysis or HPLC Analysis Data for Key Compounds

Cmpd #	Formula	Combustion Analysis or HPLC Analysis ^a Data
3	$\text{C}_{17}\text{H}_{16}\text{N}_4\text{O}_2\text{S}_2$	Method A: $t_R = 1.35$ min (90% purity). Method B: $t_R = 2.88$ min (89% purity).
4	$\text{C}_{17}\text{H}_{16}\text{N}_4\text{OS}_2$	Method A: $t_R = 1.46$ min (98% purity). Method B: $t_R = 2.98$ min (96% purity).
5	$\text{C}_{17}\text{H}_{17}\text{N}_5\text{OS}$	Method A: $t_R = 1.06$ min (95% purity).
6	$\text{C}_{17}\text{H}_{16}\text{FN}_5\text{OS}$	Method A: $t_R = 1.17$ min (98% purity). Method B: $t_R = 2.33$ min (98% purity).

7	C ₁₉ H ₁₈ FN ₅ OS	Method A: <i>t_R</i> = 1.37 min (97% purity). Method B: <i>t_R</i> = 2.48 min (98% purity).
8	C ₂₀ H ₁₉ FN ₄ OS	Method A: <i>t_R</i> = 1.58 min (97% purity).
9	C ₂₁ H ₂₂ FN ₅ OS	Method A: <i>t_R</i> = 1.49 min (98% purity). Method B: <i>t_R</i> = 2.95 min (97% purity).
10	C ₂₃ H ₂₀ FN ₅ OS	Method A: <i>t_R</i> = 1.48 min (98% purity). Method B: <i>t_R</i> = 2.66 min (98% purity).
11	C ₂₂ H ₁₉ FN ₆ OS	Method A: <i>t_R</i> = 1.10 min (98% purity). Method B: <i>t_R</i> = 2.66 min (98% purity).
12	C ₂₂ H ₂₅ FN ₆ O ₂ S	Method A: <i>t_R</i> = 0.95 min (98% purity). Method B: <i>t_R</i> = 1.83 min (99% purity).
13	C ₁₇ H ₁₅ F ₂ N ₅ OS	Method A: <i>t_R</i> = 1.25 min (97% purity). Method B: <i>t_R</i> = 2.40 min (98% purity).
14	C ₁₉ H ₁₇ F ₂ N ₅ OS	Calc'd: C, 56.84; H, 4.26; F, 9.46; N, 17.44, S, 7.98. Found: C, 56.78; H, 4.17; F, 9.60; N, 17.39; S, 8.05.
16	C ₉ H ₇ N ₃ OS	Calc'd: C, 52.67; H, 3.44; N, 20.47; S, 15.62. Found: C, 52.44; H, 3.18; N, 20.52; S, 15.55.
18	C ₁₀ H ₁₀ F ₂ N ₂ O	Calc'd: C, 56.60; H, 4.75; F, 17.91; N, 13.20. Found: C, 56.47; H, 4.55; F, 18.03; N, 13.21.

^a **Method A:** phenom-prime S5 C18 4.6 × 30 mm column, solvent A: 10% MeOH-90% H₂O-0.1% TFA, solvent B: 90% MeOH-10% H₂O-0.1% TFA, flow rate = 4 mL/min; linear gradient time = 2 min; start %B = 0, final %B = 100, stop time 3 min; **Method B:** phenomenex Su C18 4.6 × 50 mm column, solvent A: 10% MeOH-90% H₂O-0.1% TFA, solvent B: 90% MeOH-10% H₂O-0.1% TFA, flow rate = 4 mL/min; linear gradient time = 4 min; start %B = 0, final %B = 100, stop time 5 min.

V. Detailed Description of Pharmacokinetic Assays

Pharmacokinetic parameters obtained in mouse. To evaluate the oral bioavailability of compound **14** in male Balb/C mice, a single dose was delivered as a solution by either tail vein injection (iv, 10 mg/kg, PEG400:tween 80:water, 20:5:75, v/v) or by oral gavage (po, 90 mg/kg, PEG400:tween 80:water, 40:10:50, v/v). The mice were fasted overnight prior to dosing and fed 4 hours post dose. A

total of 18 mice were used in the study ($n = 9$ each for iv and po groups). Three serum samples were collected from each mouse, the first two samples by retro-orbital bleed ($\sim 100 \mu\text{L}/20\text{-}25 \text{ g mouse}$) and the third sample by cardiac puncture. Blood samples were collected at 0.05, 0.5, 1, 4, 6, 8, and 24 h time points following iv dosing and at 0.25, 0.5, 1, 4, 6, 8, and 24 h following oral dosing. Blood samples were allowed to clot on ice, centrifuged and then serum harvested. Serum samples were stored at -20°C until analysis. Concentrations of parent compound were later determined by LC/MS/MS. Composite serum concentration-time profiles were constructed for pharmacokinetic analysis using noncompartmental methods.

Pharmacokinetic parameters obtained in rat. To evaluate the oral bioavailability of compound **14** in male Sprague-Dawley rats, a single dose was delivered as a solution either intravenously as a 10 min infusion (iv, 10 mg/kg, PEG400:tween 80:water, 20:5:75, v/v) or by oral gavage (po, 50 mg/kg, PEG400:tween 80:water, 40:10:50, v/v). The rats were fasted overnight prior to dosing and fed 4 hours post dose. A total of 6 rats were used in the study ($n = 3$ each for iv and po groups). Blood samples were collected at 0.17, 0.25, 0.5, 0.75, 1, 2, 4, 6, 8, 10, 24, and 48 h time points following iv dosing and at 0.25, 0.5, 0.75, 1, 2, 4, 6, 8, 10, 24, and 48 h following oral dosing. Approximately 0.3 mL of blood was collected from the jugular vein in EDTA-containing tubes, and plasma was obtained by centrifugation. Plasma samples were stored at -20°C until analysis. Concentrations of parent compound were later determined by LC/MS/MS. Pharmacokinetic parameters were calculated by noncompartmental methods.

Pharmacokinetic parameters obtained in cynomolgus monkey. To evaluate the oral bioavailability of compound **14** in male cynomolgus monkeys, a single dose was delivered as a solution either intravenously as a 10 min infusion (iv, 1 mg/kg, PEG400:water, 30:70, v/v) or by oral gavage (po, 5 mg/kg, PEG400:tween 80:water, 40:10:50, v/v). The monkeys were fasted overnight prior to dosing and fed 4 hours post dose. A total of 3 monkeys were used in the study utilizing a crossover study design. Blood samples were collected at 0.17, 0.25, 0.5, 0.75, 1, 2, 4, 6, 8, 10, and 24 h time points

following iv and po dosing. Approximately 1 mL of blood was collected in EDTA-containing tubes, and plasma was obtained by centrifugation. Plasma samples were stored at -20°C until analysis. Concentrations of parent compound were later determined by LC/MS/MS. Pharmacokinetic parameters were calculated by noncompartmental methods.

VI. References

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